Supplementary Information

Breast Cancer Cell Secretome Analysis to Decipher miRNA Tumor Biology and Discover Potential Biomarkers

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Figure S1

A

Protein Domains in MCF7-miR526b and MCF7-miR655 Secretome Threshold			
Node Color	**Protein Domain**	**Number of Nodes (n=39)**	**P-value**
	C-terminus of histone H2A	13	8.12e-26
	Septin	5	2.26e-9
	14-3-3	4	3.93e-8
	Thioredoxin	3	7.50e-4

B

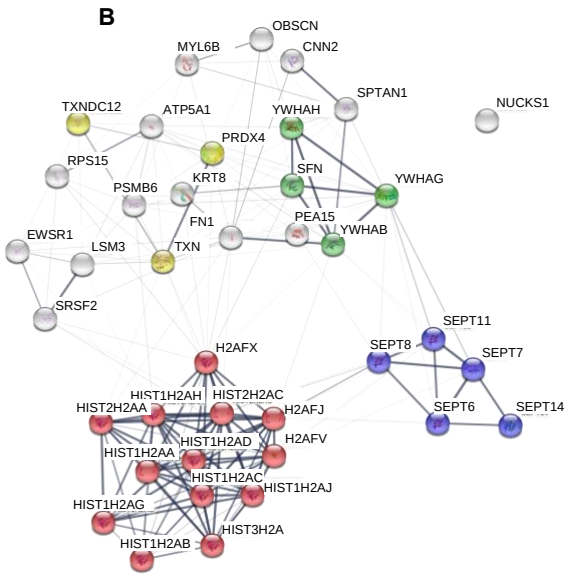

Figure S1. Differently secreted miRNA-high protein-coding genes protein domains at the first step of data curation (**A**) Significantly enriched protein domains within our secretome threshold. (**B**) Protein-protein interaction network of protein-coding genes within our secretome threshold. Colored nodes indicate protein domain from the adjacent table. Thicker gray lines between nodes indicate stronger interactions.

Figure S2

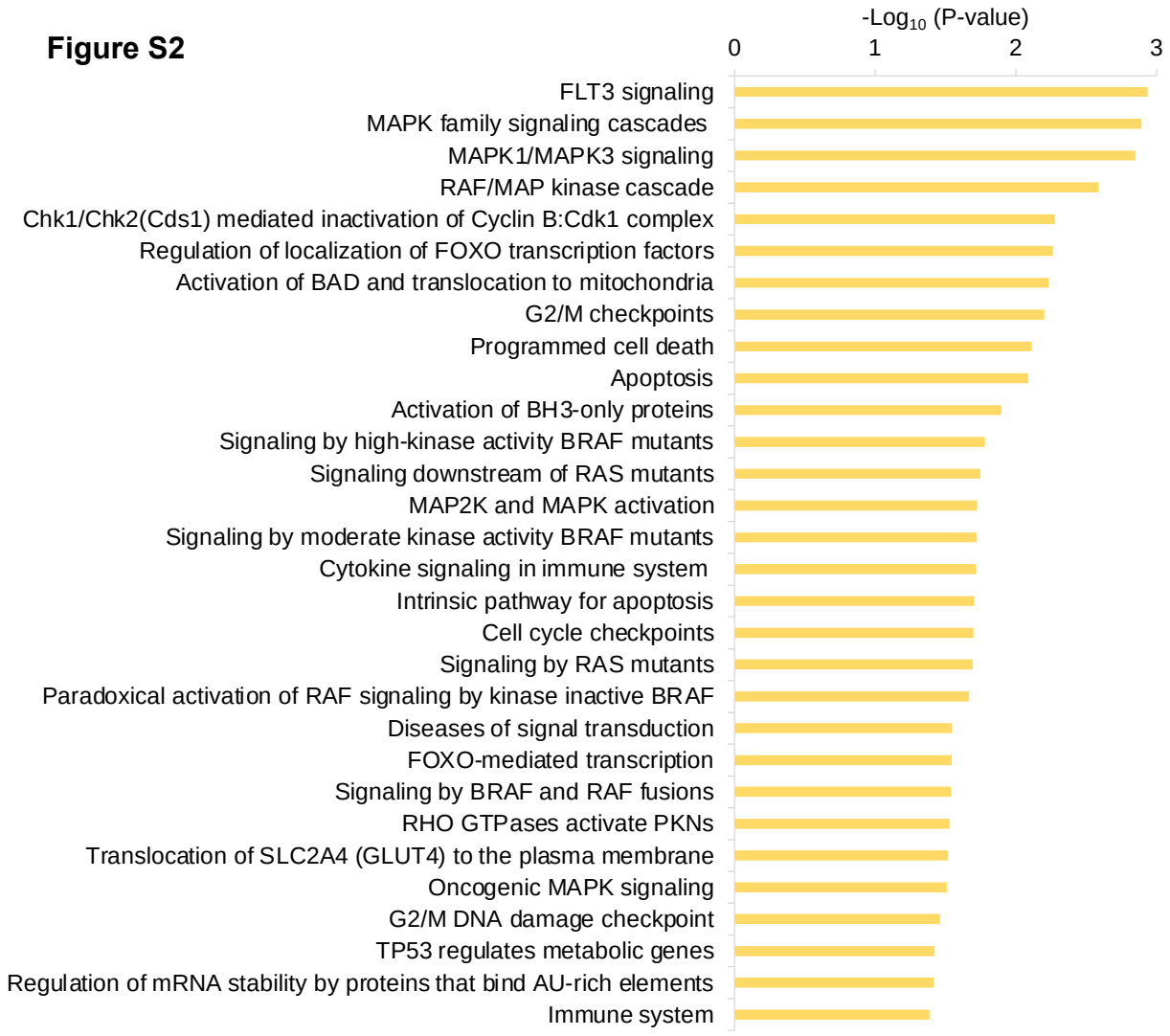

Figure S2. Secretome markers significantly enriched pathways.

Figure S3

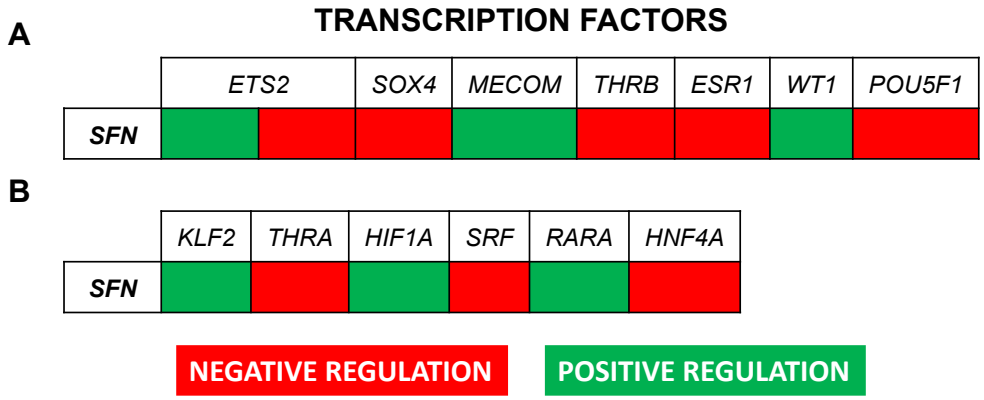

Figure S3. miR526b or miR655 targets matched with *SFN* TFs explaining miR526b or miR655 secretome expressions. Regulatory effects of TFs on *SFN* for (**A**) miR526b and (**B**) miR655.

Table S1

Gene Name (Secretome Expression)	General Function	Cellular Component GO	Biological Process GO	Molecular Function GO	Association with Breast Cancer (BC)
YWHAB (↑)	• Regulates cell cycle machinery and signaling pathways • Binds to many partners, modulating the activity of the binding partner	Cytosol (68), cytoplasm (5), extracellular exosome (2), membrane (2), focal adhesion (1), melanosome (1), vacuole (1), vacuolar membrane (1)	Cellular protein organization (1), cytoplasmic sequestering of protein (1), negative regulation of G protein-coupled receptor signaling pathway (1), negative regulation of protein dephosphorylation (1), positive regulation of catalytic activity (1), protein targeting (1) signal transduction (1)	Binding: Protein (109), histone deacetylase (2), identical protein (2), protein domain specific (2) cadherin (1), enzyme binding (1), phosphoprotein (1). Protein kinases inhibitor activity (1)	Upregulation • In MCF7 circulating BC cells • Leads to decreased BC survival • In non-triple-negative (TN) BC cell lines and patients Downregulation • In MCF7 BC cells • In TNBC
TXNDC12 (↑)	• ER stress defence • Catalyzes disulfide bond formation • Similar to protein-disulfide isomerases	Endoplasmic reticulum (3), endoplasmic reticulum lumen (2)	Negative regulation: of cell death (1) and endoplasmic reticulum stress-induced intrinsic apoptotic signaling pathway (1)	Protein binding (12), protein-disulfide reductase activity (3), and oxidoreductase activity (1)	• Not directly associated with BC • However, two subfamily members, *AGR2* and *AGR3*, have close similarity, and are upregulated in BC tumors and correlated with ER status • Family member, *TXNDC5,* upregulated in BC
MYL6B (↑)	• Cellular motor protein	Cytosol (5), myosin complex (2), extracellular exosome (1), muscle myosin (1), unconventional myosin (1)	Muscle contraction (1), muscle filament sliding (1), skeletal muscle tissue development (1)	Protein binding (5), structural constituent of muscle (2), calcium ion binding (1), cytoskeletal motor activity (1)	• Not directly associated with BC • Family member, *MYO10*, upregulated in two different BC datasets, and mediates adhesion, migration, invasion, and metastasis of BC cells in vitro and in vivo
SFN (↑)	• Regulates cell cycle and signaling pathways • Binds to many partners, usually modulating the activity of the binding partner	Cytosol (11), cytoplasm (4), nucleus (3), extracellular exosome (2), extracellular region (2), extracellular space (1)	Establishment of skin barrier (2), positive regulation of epidermal cell division (2), regulation of epidermal cell division (2), signal transduction (2), cellular protein localization (1), intrinsic apoptotic signaling pathway in response to DNA damage (1), keratinization (1). Keratinocyte: development (1) and differentiation (1). Negative regulation of: cysteine-type endopeptidase activity involved in apoptotic process (1), keratinocyte proliferation (1) and protein kinase activity (1) Positive regulation of cell growth (1) and protein export from nucleus (1). Regulation of: cell cycle (1) and cyclin-dependent protein serine/threonine kinase activity (1). Skin development (1)	Binding: Protein (75), identical protein (2), protein kinase (2), cadherin (1), and phosphoprotein binding (1). Protein kinase C inhibition (1)	Upregulation • In nuclear and cytoplasmic SFN in metastatic and primary BC • In stroma of metastatic and primary BC tumors • Reduces survival of BC patients and predicts which patients develop metastasis in basal-like BC • In patients had worse overall survival • In drug resistant cells MCF7 cells • Tracks the malignant phenotype in basal-like BC and is associated poor survival • In BC and can be used as therapeutic target Downregulation • In MCF7 and MDAMB231 BC cells • Promotes HER2-induced tumorigenesis • Occurs during BC tumorigenesis in vivo Methylated • In BC and suitable blood-based BC biomarker • At SFN locus leads to gene silencing in BC
FN1 (↓)	• Involved in cell adhesion, cell shape, motility and migration processes including embryogenesis, wound healing, blood coagulation, host defense, and metastasis • Both anastellin (*FN1* fragment) and superfibronectin (*FN1* polymer) inhibit tumor growth, angiogenesis, and metastasis	Extracellular region (35), collagen-containing extracellular matrix (13), plasma membrane (11). Extracellular exosome (3), space (3), matrix (2). Apical plasma membrane (1), basement membrane (1), blood microparticle (1), endoplasmic reticulum lumen (1), endoplasmic reticulum-Golgi intermediate compartment (1) fibrinogen complex (1)	Biological process involved in interaction with symbiont (4), cell adhesion (3), substrate adhesion-dependent cell spreading (3), cell-matrix adhesion (2), cell-substrate junction assembly (2), negative regulation of monocyte activation (2), acute-phase response (1), angiogenesis (1), blood coagulation (1) calcium-independent cell-matrix adhesion (1), endodermal cell differentiation (1), heart development (1), integrin-mediated signaling pathway (1), negative regulation of transforming growth factor beta production (1), nervous system development (1). Positive regulation of axon extension (1), cell population proliferation (1), fibroblast proliferation (1), gene expression (1), peptidase activity (1), phosphatidylinositol 3-kinase signaling (1) and substrate-dependent cell migration, cell attachment to substrate (1). Regulation of cell shape (1), ERK1 and ERK2 cascade (1) and protein phosphorylation (1). Response to wound healing (1), and wound healing (1)	Protein binding (67), extracellular matrix structural constituent (9). Binding: Identical protein (5) enzyme (4), integrin (4), signaling receptor (4), protease (3), collagen (2) heparin (2), proteoglycan (2), chaperone (1), disordered domain specific (1). Peptidase activator activity (1), protein C-terminus binding (1)	Downregulation • Inhibits BC metastasis • Allows mesenchymal BC cells to regain epithelial characteristics and initiate tumor growth Upregulation • Serum-based BC biomarker • Enhances cell migration, invasion and EMT in MCF7 cells • TME expression induces DNA demethylation and initiates changes in histone marks activating metastatic gene (*MMP2)* in MCF7 cells • Activates PI3K/AKT pathway • Lower BC survival • Affects metastasis, tumor/stromal cell migration, growth, and angiogenesis and is a potential BC therapeutic target • Correlates with advanced BC staging and poor clinical outcomes • Associated with the prognosis of lymph node negative BC patients
PSMB6 (↓)	• Involved in the proteolytic degradation of most intracellular proteins • Postacidic (peptides bond hydrolysis occurs directly after acidic residues)	Cytosol (54), nucleoplasm (8), proteasome core complex (6), nucleus (3), cytoplasm (2), proteasome complex (2), extracellular exosome (2)	Proteasomal protein catabolic process (1), proteolysis (1), proteolysis involved in cellular protein catabolic process (1)	Protein binding (5), threonine-type endopeptidase activity (2), cadherin binding (1). Endopeptidase (1), hydrolase (1), and peptidase activity (1)	• *PSMB6* and *PSMB1* are downregulated in BC patients, while other eight *PSMB* subunits upregulated • *PSMB6* was the only *PSMB* subunit not significantly higher in BC patients
PRDX4 (↓)	• Antioxidant enzyme • Catalyzes H_2O_2 and hydroperoxides to water and alcohols • Cell protection against oxidative stress • Sensor of H_2O_2-mediated signaling events • Regulates NFκB activation	Endoplasmic reticulum (4) Cytoplasm (3) Cytosol (2) Extracellular region (2) Extracellular exosome (1) Ficolin-1-rich granule lumen (1) Nucleus (1) Secretory granule lumen (1)	Cellular oxidant detoxification (6) Cell redox homeostasis (2) Extracellular matrix organization (1) I-kappaB phosphorylation (1) Male gonad development (1) Negative regulation of male germ cell proliferation (1) Peptidyl-proline hydroxylation to 4-hydroxy-L-proline (1) Protein maturation by protein folding (1) Reactive oxygen species metabolic process (1) Response to oxidative stress (1) Spermatogenesis (1)	Protein binding (8) Antioxidant activity (2) Oxidoreductase activity (2) Peroxidase activity (2) Identical protein binding (1) Thioredoxin peroxidase activity (1)	• Decreased expression has worse BC survival, and *PRDX4* acts as response to increased reactive oxygen species in BC tissue • Secreted at high levels in MCF7 and MDAMB231 BC cells and may be a viable BC therapeutic target • Overexpression significantly correlated with survival in BC patients, showing therapeutic target and BC biomarker potential • mRNA overexpression significantly associated with poor overall survival in BC patients • Significantly upregulated in TNBC
PEA15 (↓)	• Negative regulator of apoptosis	Cytoplasm (2) Cytosol (2) Microtubule associated complex (1) Nucleoplasm (1)	Negative regulation of extrinsic apoptotic signaling pathway via death domain receptors (3) Apoptotic process (1) Carbohydrate transport (1) Negative regulation of glucose import (1) Positive regulation of extrinsic apoptotic signaling pathway via death domain receptors (1) MAPK cascade (1) Regulation of apoptotic process (1) Response to morphine (1)	Protein binding (7)	• Downregulation linked with less invasion and aggressiveness in BC • Reduced protein expression in metastatic BC cells • Downregulated in BC samples • Downregulation promotes EMT/tumorigenesis in TNBC • Potent non-viral vector for therapeutic application in BC • Downregulation reduces cell growth, cell number and viability • PEA15 phosphorylation is necessary for BC tumor cell formation and functions as a BC tumor suppressor. However, PEA15 phosphorylation changes its binding partners, converting PEA15 to an oncogene

Table S1. Individual GOs of secretome markers.

Table S2

Markers	Identified in Human Plasma?	Protein Evidence Level	Protein Evidence
YWHAB	Yes	1: Protein level	Canonical
TXNDC12	Yes	1: Protein level	Canonical
MYL6B	Yes	1: Protein level	Canonical
SFN	Yes	1: Protein level	Canonical
FN1	Yes	1: Protein level	Canonical
PSMB6	Yes	1: Protein level	Canonical
PRDX4	Yes	1: Protein level	Canonical
PEA15	Yes	1: Protein level	Canonical

Table S2. Secretome markers found in human plasma.